CHROMATIN BRIDGES AND IRREGULARITY OF MITOTIC COÖRDINATION IN THE PENTATOMID PEROMATUS NOTATUS AM. AND SERV.

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Introduction

A specimen of the pentatomid species *Peromatus notatus* obtained in 1937 presents such significant modifications of the orthodox course of meiosis, that a description and consideration of the most striking features seem warranted. As will be seen, the individual in question clearly is an exceptional case, but its departure from the normal is based on fundamental changes that have altered its mitotic mechanism in a very definite way. Apparently it is chiefly the relative timing of the various mitotic processes that has been affected, and the chromosomes and the spindle apparatus are, so to speak, out of step with each other. Their behavior under these conditions is of some interest in the analysis of mitosis in general.

Material

The specimen was caught on Barro Colorado Island in the Panama Canal Zone, in March, 1937. Its testes were fixed in B 15 within two hours of capture. It was close to the maximum size recorded for the species, in good condition, and very active.

Peromatus notatus, like the other six species of the genus, is strictly neotropical in its distribution. Examination of the sixteen specimens in the collections of the U. S. National Museum and the American Museum of Natural History shows that the species is subject to considerable variation in form and color. Variability in form is, however, more or less superficial and chiefly due to differences in the size and shape of the pronotal spines. The usual chestnut-brown color is replaced by green in some individuals from Panama (identified and labeled in the American Museum collection by H. G. Barber). It is a specimen of the latter type caught on Barro Colorado Island in 1941 that has served for comparison in the present study. It offers a typically pentatomid spermatogenesis which is almost indistinguishable from that of a specimen of *Peromatus truncatus* obtained in the same locality.

Spermatogonia

The spermatogonial divisions of the exceptional individual show no unusual features. The spindles conform to the common type, the chromosomes divide normally, and successive spermatogonial cell generations show no variation in chromosome number. The latter comprises the usual set of 14 chromosomes, in which one pair is a little larger and one pair somewhat smaller than the rest. The X is intermediate in size, whereas the Y is about as large as a member of the smallest pair (Fig. 1).

Meiotic Prophases to Diakinesis

Up to late diakinesis, the meiotic prophase stages conform to the usual pentatomid behavior. The sex chromosomes are heteropycnotic and frequently, though not always, appear joined from leptotene to diakinesis. There is a plasmosome which dwindles rapidly after the pachytene stage.

It is not until toward the end of diakinesis that the first unusual feature is encountered. Just as in other pentatomids, the two centers at this time move toward opposite sides of the nucleus. Both are in contact with the nuclear membrane and when they have reached their final position, the membrane underneath them is pulled or bulged outward.

This and the oval form of the nucleus, assumed in the direction of the centriolar axis, have frequently been noted (as early as 1891 by Henking). The point to be noted in this instance, however, is that the centers in the majority of cases are not on truly opposite points of the nucleus but are closer to each other on one side than on the other (Fig. 2). It is, of course, true that in other pentatomids also the position of the centers is not always geometrically exact, but the position here clearly is not accidental. This is borne out by the metaphase conditions that immediately follow the breakdown of the nuclear membrane.

The First Metaphase

The equator of the first spindle is in almost all cases displaced to one side, so that a line through the two centers does not represent the symmetrical axis of the mitotic figure as in other cases. In many cells all the chromosomes form a plate that lies to one side of the centriolar axis and hence the half spindle components are similarly displaced (Fig. 4). A few continuous fibres can sometimes be seen to stretch between the centers without such displacement, indicating their relative independence of the chromosomes. The latter rarely form a circle or round

plate, but constitute a semicircle with the two sex chromosomes usually but not always lying on the concave side (Fig. 3).

Despite this distortion of the mitotic apparatus, the tetrads divide in orderly fashion (Fig. 6) and the sex chromosomes undergo an equation division, just as in the normal *Peromatus* and other pentatomids. The peculiar configuration of the chromosome plate, however, is mirrored in the two daughter groups and may persist until middle anaphase (Fig. 5).

The initial movement of the dyads seems to occur without reference to the center and hence shows no effect of their askew position (Fig. 4). This is, of course, what might be expected since in nearly all cases known these first division stages of the chromosome appear to be autonomous.

The configuration of this first spermatocyte spindle challenges several interpretations concerning the mitotic mechanism. If the poles of the spindle are established by a mutual repulsion of two centrioles, it is very difficult to conceive of anything but a symmetrical spindle structure resulting therefrom. If the chromosomes assume their metaphase position because they react to forces from the poles, it is again not easy to understand why they should take such an "off center" position as they do. The conclusion is unavoidable that the mitotic conditions are affected by factors which normally are not present at this time.

First Anaphase to Second Anaphase

In the normal *Peromatus* as well as in most other pentatomids so far investigated, each of the centers carries two centrioles already at diakinesis. These two centrioles usually remain closely associated until telophase, though occasionally they have separated by some 15° before the end of anaphase (see, for instance, Paulmier's Fig. 29, 1899). The movement is quickened at telophase and before the second division is begun, the two centrioles are separated by 180°. There appears to be no exception to the rule that in Heteroptera the polar axis of this second division is at right angles to that of the first. This relation is especially striking in those cases where the interzonal connections of the first division continue to stain intensely, as in *Pachylis* (Fig. 8, and also those of other Heteroptera by Henking, 1891; Montgomery, 1898; and Paulmier, 1899).

The course followed in the present case is characterized by either one of two departures from the normal procedure just described. In about 75 per cent of the cells there is a marked precocity in the movements of the centrioles. Starting with little more separation than in normal cases, they diverge quickly after the early anaphase and in most cases have separated by 40°–45° before the anaphase movement of the chro-

mosomes has been completed (Fig. 7). Among the remaining cells about half show no such precocious separation of the centrioles, but the center as a whole may shift as much as 90° from the axial position of the first division (Fig. 9). In short, in such cases both centrioles assume the position of one of the poles of the second division, though the chromosomes are still in late anaphase of the first.

It was a matter of some surprise to find that in every such instance both centers moved to the same side of the anaphase cell. But this may simply be the consequence of the asymmetry of the first spindle which puts both centers closer to one side than the other to begin with. The two extremes of centriolar behavior are bridged by intermediate conditions which are not always easy to interpret. Thus the centrioles may succeed in separating after the center as a whole has begun to shift, or else one of the centrioles is for some reason held at the first pole and only the other moves toward its position for the second division (Fig. 7).

Whatever the type of variation may be, one point is held in common by all these cells. The processes that establish the achromatic figure of the second division are decidedly in advance of the corresponding steps in normal cells.

The precocity of the centers has marked effects on the behavior of the chromosomes. This is, perhaps, no more than might be expected, since they are still in the anaphase of the first division when the centers are already in process of establishing the mechanism for the second. The chromosomes show a definite response to the two poles which is manifested most strikingly in a tendency to divide again at this early

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PLATE I

Drawings made with Zeiss, 90 × objective and 20 × ocular. They were reduced ⅙ in reproduction.

FIG. 1. Three plates showing the 14 spermatogonial chromosomes.

FIG. 2. Diakinesis. The two centers are closer to each other on one side than the other.

FIG. 3. First metaphase. Autosomal tetrads arranged in semicircle, with *X* and *Y* on the inside.

FIG. 4. Side view of early anaphase, showing asymmetrical spindle. The autonomy of the initial separation of chromosomes is attested by lack of orientation toward the centers.

FIG. 5. Polar view of two sister groups in first anaphase, still showing typical arrangement.

FIG. 6. Middle anaphase of first division. The centrioles at each pole are separated less than usual.

FIG. 7. Upper pole of a late anaphase of first division. The two centrioles already have separated by about 45°, and there is no collocation of the chromosomes.

FIG. 8. Interphase in the coreid *Pachylis*, to show the characteristic relation of the second to the first spindle in the Heteroptera.

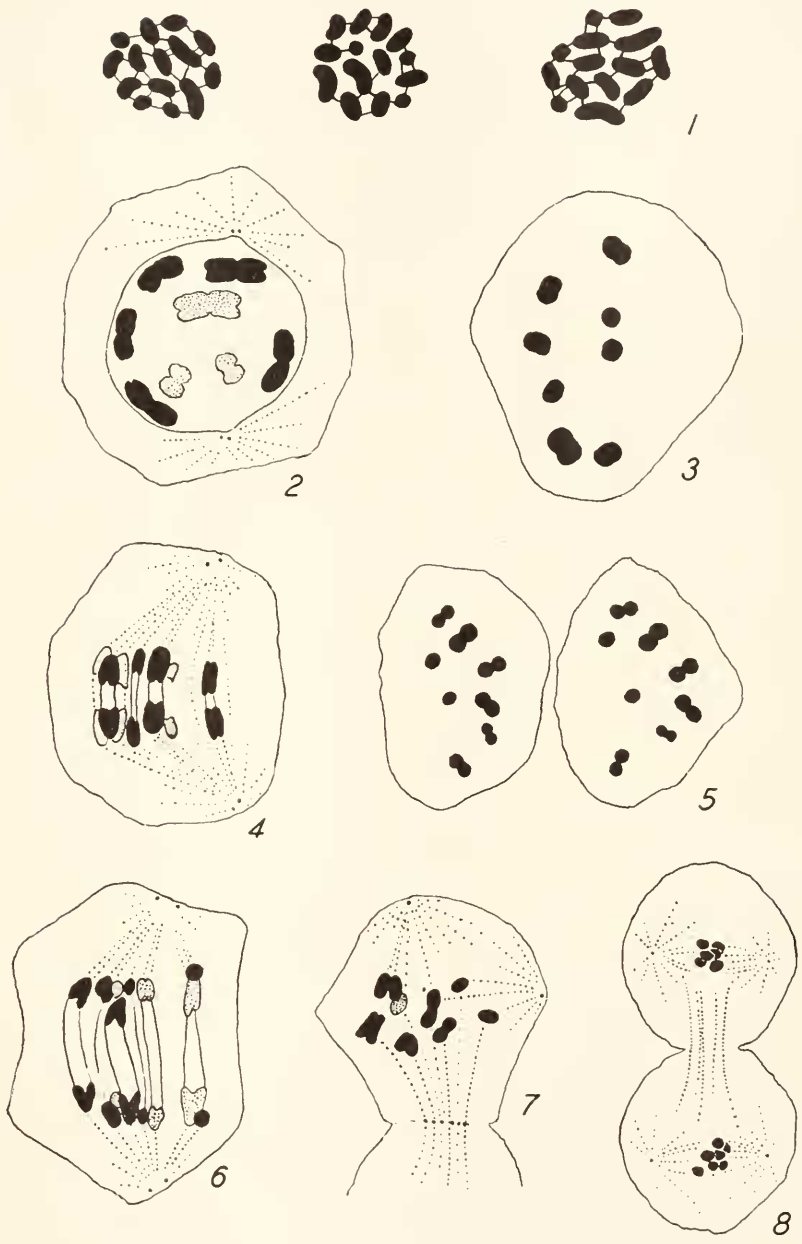

PLATE I

stage (Fig. 10). If this occurs before they have become dissociated from the interzonal connectives, such peculiar configurations as shown in Fig. 12 may result. In these as well as in less extreme cases the significant feature lies in the marked elongation of the chromosomes.[1]

This occurs despite the fact that the two chromatids of each dyad move in opposite directions toward the centrioles which are establishing a new axis. In other words, though the demarcation between the two chromatids is clearly indicated—as indeed it already is in diakinesis—and though the attenuation of the chromatids evidently betokens forces that tend to move them apart, they do not succeed in separating from each other (Figs. 10–13). The attenuating process continues until the chromosome body is torn into two pieces. The break apparently occurs at random and usually not in the natural line of separation between the chromatids (Figs. 13 and 14). Hence the amount of chromosome material distributed to each pole is variable and certainly not normal.

During this time the centriolar movement is completed. As a result the flexion that characterizes the spindles during the early part of this division disappears and the spindles of the late second anaphase are perfectly straight (Fig. 13).

PLATE II

FIG. 9. Late anaphase of first division. Each of the centers (both show two centrioles) has moved through 90° toward one pole of the second division.

FIG. 10. Centrioles of second division acting on chromosomes which are still in the condition of the first anaphase. (In Figs. 10, 11, and 12 only one of two sister cells is shown.) The demarcation between the chromatids is evident in several dyads.

FIG. 11. Second division showing attenuation, with chromatid demarcation showing in several dyads.

FIG. 12. Second division. The centrioles have separated relatively little, and the whole figure is strongly flexed as a result. Trace of interzonal connections of first division still showing at lower left.

FIG. 13. Late anaphase of second division. The spindle has straightened out. Chromatid demarcation still present in two of the dyads.

FIG. 14. Telophase of second division. There is no trace of collocation. The abnormality of the chromosome division is evident.

FIG. 15. Late telophase. Chromosomes still scattered and already becoming diffuse.

FIG. 16. Spermatid with four micronuclei, one Nebenkern, and one tail filament.

[1] It will be seen that the side of the chromosome presented toward the pole in the first division does not correspond to that of the second. This puzzling feature is, however, encountered in all Heteroptera and does not constitute a peculiarity of the present case. The explanation may lie in the fact that in the Hemiptera we are dealing with a "diffuse" instead of a localized kinetochore, as Hughes-Schrader and Ris (in press) have recently established.

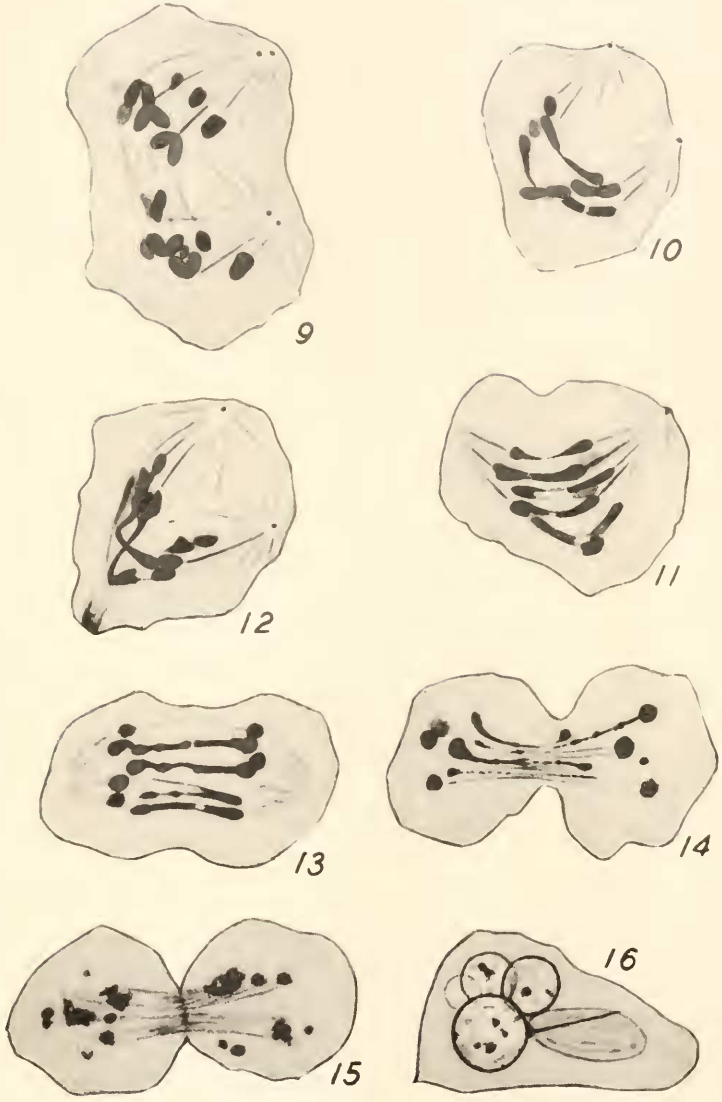

PLATE II

Second Telophase to Spermatozoa

Since the chromosomes of the first division are subject to the forces of the second division while they are still in anaphase, nothing can be said of their behavior under telophase conditions. Since there is only one centriole at each pole of the second division, no comparable centriolar disturbance takes place there and the chromosomes reach the telophase in every case.

Instead of the collocation of chromosomes that is typical of normal telophases, the chromosomes here actually tend to move further apart or to repel each other (Fig. 14). This tendency is not overcome even by the time that the chromatin becomes diffuse and as a result the products of the division lie more or less scattered in the cell (Fig. 15). Separate, small nuclei are formed from such masses of chromatin, and the spermatid is always a multinucleate cell (Fig. 16). In most cases only one Nebenkern is formed though in some instances two have been encountered. In no cell, however, does one find more than one axial filament and middle piece. These are associated with one of the nuclei which is not necessarily the largest one.

Apparently even the smallest of the nuclei takes steps toward the elongation that characterizes the formation of the sperm head. Later, however, there is much degeneration, though some of the sperms appear more or less normal.

The relative independence of mitotic phenomena in the cytoplasm and in the nucleus is attested by the fact that all the manoeuvers of the centers and the chromosomes do not hinder the division of the cytoplasm. Separate and complete cells, more or less equal in size, are found both after the first as well as the second division.

Discussion

The relationship of the significant features of this case is not always entirely clear, though it seems safe to assume that they are interconnected. They may be listed as follows: 1. The asymmetry of the first division figure. 2. The precocity in the behavior of the centers. 3. The attenuation and irregular division of the chromosomes in the second division. 4. The formation of multinuclear spermatids.

(1). The asymmetry of the first division figure is difficult to explain. If bipolarity is brought about merely by a mutual repulsion of two centers, the latter should be separated by 180° on the diakinetic nucleus and in the first metaphase. Again, the location of the chromosome plate, if it rests on a system of repulsive or attractive forces correlated with

those of the centers, should be on the axis formed by the latter. To explain the askew position of the chromosomes, it might be suggested that a primary spindle, comprised of fibres extending from pole to pole, arises before the chromosomes have formed a metaphase plate. This spindle then constitutes a core into which the chromosomes do not penetrate and hence they are disposed in the form of a semicircle around it. But such a hypothesis does not touch the root of the matter, which lies in the asymmetrical position of the centers themselves. And for this nothing more can be said than that a factor or force, probably extraneous to centers and chromosomes, is responsible.

(2). The extreme degree of separation of sister centrioles during the first division is clearly an indication of precocity in their cycle. Not so pertinent to this conception are those instances where the entire center, including both centrioles, moves to one of the poles of the second division (Fig. 9).

This might be attributed to the elongation of the spindle which pushes both centers around the periphery to one side. Precocity would there be expressed only in the development of astral rays and half-spindle fibres which actually appear to be growing at a time when in normal cases they are waning.

If, however, the movement of the undivided center is not thus accidental, its shift to the axis of the second division must mean that this pole is predetermined. This would imply that the centers are only secondarily concerned. The evidence hardly permits of extensive hypothetical considerations, but the early establishment of such a pole might involve forces that also are responsible for the asymmetry of the first division.

(3). But whether or not the centers are the primary agents in the determination of polarity, their direct influence on the chromosomes is not to be denied. This is strikingly shown in the premature second division, where it appears that the precocity of the centriolar processes is correlated with an exertion of forces that are normally not in evidence until a later stage. Their influence is indicated by the fact that the mitotic movement of chromosomes is toward the two centers from the very start. The autonomous separation of chromatids which takes place without reference to centers and which always comprises the first step under normal conditions, does not take place at all.

The attenuation of the chromosomes suggests that they are subjected to tensile forces. The failure of the chromatids to dissociate from each other under such conditions must then indicate that they are not yet completely ready when the centriolar forces are exerted thus precociously.

The lag does not lie in the chromosome proper, for in this as well as in normal cases all the chromatids are sharply demarcated from each other already in the preceding diakinesis (Fig. 2). That this demarcation persists into the second division is clearly shown in Figs. 10, 11 and 13, and the conclusion hence is unavoidable that a separation is prevented by other factors. The latter can be sought only in either the sheath or the matrix of the chromosomes, and it is therefore this constituent which is not yet ready for the division and holds the chromatids together.

It may be pointed out that the attenuation of chromosomes during division is not at all rare and that its cause is by no means always the same. It has been reported in cells that were subjected to X-ray or radium treatment. It is then usually correlated with a tendency of chromosomes to clump, and secondarily to translocations and inversions. Such cases are difficult to analyze since so many of the mitotic processes seem to be affected.

It has been described in tapetum cells (Steil, 1935) which show signs of degeneration. The attendant irregularities may well arise from an upset in the timing of the various mitotic processes as in the present case, but the necessary details of behavior that would justify such a conclusion are not available.

Bauer (1931) has reported it in *Tipula* and ascribes it to the presence of supernumeraries. The disturbance is there correlated with an adhesion of the chromosomes to each other.

It results from changes in the physical condition of the chromosomes, which in at least one case arise from the mutation of a single gene (Beadle, 1932). The "stickiness" which there characterizes the chromosomes seems to be caused chiefly by changes in the matrix and it is not impossible that the frequent attenuation during division is closely akin to that observed in *Peromatus*.

Lastly, it is a well-recognized characteristic of chromosome inversions which have resulted in dikinetic or dicentric chromosomes. Such "chromosome bridges" have played so striking a rôle in recent cytogenetic investigations that there has been a tendency to forget that not all chromatin bridges need be of the same nature. Thus Gentscheff and Gustafsson (1940) in their excellent study of meiosis in *Hieracium* utilize Beadle's conception that fragmentation of his maize chromosomes results from changes in the matrix, but quite ignore his explanation that his chromosome bridges were due to stickiness and increase in viscosity. Instead, they ascribe the very similar bridges in *Hieracium* to inversions and thus seem to agree with Darlington (1937, p. 320), who does not accept Beadle's convincing interpretation and states that "at anaphase

several bridges are found, showing that the changes include inversions."

It need hardly be pointed out that in the present case of *Peromatus notatus,* an explanation that rests on inversions is not tenable at all. This is already strongly indicated by the fact that the first division shows no bridges whatever, whereas they characterize all second divisions. To explain this on the basis of inversions would necessitate that two crossovers of a very specific type take place, and that these occur in the meiotic prophase of all cells. This would, moreover, result in a chromosome fragment which most assuredly is not present. Further, such a hypothesis would assume an orthodox, localized kinetochore, whereas here we are dealing with one of the diffuse type (Hughes-Schrader and Ris, in press). Finally, it must be remembered that an inversion bridge arises because bipolar tension is exerted upon a portion of a chromosome which does not include the natural line of demarcation between chromatids and which therefore can be divided only by tearing. In contrast, the bridges in *Peromatus* include the region where two chromatids, sharply demarcated from each other, are placed end to end. Dissociation therefore should and would follow quite normally without attenuation, if it were not hindered by the matrix or the sheath.

(4). Multinucleate spermatids arise because of the upset in the timing of mitotic processes. The chromosomes of the second division arrive at telophase when, in a sense, they are still in the anaphase condition. The mutual repulsion that characterizes them at the normal metaphase and anaphase, is therefore still encountered here when they have arrived at the poles. Hence there is the reverse of the usual collocation, the chromosome bodies are scattered singly or in small groups through the cell, and several micronuclei are found. The case for an irregularity in the timing of the centers is further supported by the fact that the actual division of the centrioles, albeit their movements are precocious, is in itself quite normal and only one middle piece and one axial filament are encountered in every multinucleate spermatid.

Conclusion

The nature of the case makes it rather futile to speculate on the origin of the meiotic abnormalities just described. Practically nothing is known about the ecology of the genus, and the possibility of interracial and interspecific crosses is purely hypothetical.

Clearly, however, the case is an exceptional one for the species. The conditions basically affect the production of normal sperms and can have no survival value. Indeed, the rather orthodox course of spermatogenesis in other specimens of *Peromatus* renders this certain.

But so far as this individual is concerned, the abnormality is a deep-seated one since the absence of normal spermatids indicates that it has persisted for some time. The conditions strongly suggest that at least one of the mitotic processes has fallen out of step and that coördination with the other processes becomes progressively more difficult in the successive cell generations, from spermatogonia to spermatids. The disturbance has no visible effect on the spermatogonia; has a well-defined influence on the spindle mechanism of the first division without, however, upsetting the essential aspects of orderly chromosome division; renders impossible a normal distribution of chromosomes in the second division; and culminates in spermatids that are definitely abnormal.

Summary

1. The abnormal course of meiosis in a specimen of *Peromatus notatus* is characterized by a series of well-defined irregularities.

2. The spindle of the first division shows both centers to one side of the geometrical axis and the metaphase plate displaced to the opposite side.

3. Before the chromosomes of the first division have reached the poles, they are subjected to the forces involved in the second division.

4. The effect on the chromosomes is to attenuate them without bringing about a normal division. The resulting configurations simulate inversion bridges, though that is quite clearly not their nature.

5. The spermatids receive varying amounts of chromosome material and are multinucleate.

6. It is suggested that this abnormal meiosis is due to an irregularity in the timing of one of the mitotic processes. The indications are that this process involves the movement of the centers.

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